Claims

1. A method of making a polysaccharide over-producing bacterium comprising

introducing into a bacterium an *ica* nucleic acid operably linked to an *ica* regulatory nucleic acid,

wherein the *ica* regulatory nucleic acid comprises

(a) nucleic acid molecules which hybridize under stringent conditions to a nucleic acid molecule having a sequence of SEQ ID NO:2, have an addition, deletion or substitution in a region between and including nucleotides 9 and 43 of SEQ ID NO:2, and that enhance production of a polysaccharide from an *ica* locus, and

(b) complements thereof.

2. The method of claim 1, wherein the bacterium is a *Staphylococcus* bacterium.

3. The method of claim 2, wherein the *Staphylococcus* bacterium is selected from the group consisting of *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Staphylococcus capitis*, *Staphylococcus caprae*, *Staphylococcus hemolyticus*, *Staphylococcus auricularis*, *Staphylococcus intermedius*, *Staphylococcus lugdunensis*, *Staphylococcus pasteuri*, and *Staphylococcus piscifermentans*.

4. The method of claim 1, further comprising measuring polysaccharide production from the bacterium, wherein a high level of polysaccharide production is indicative of a polysaccharide over-producing bacterium.

5. The method of claim 1, wherein the *ica* regulatory nucleic acid comprises the nucleotide sequence of SEQ ID NO:1.

6. The method of claim 1, wherein the *ica* regulatory nucleic acid comprises the nucleotide sequence between and including nucleotides 9 and 38 of SEQ ID NO:1.

7. The method of claim 1, wherein the *ica* regulatory nucleic acid comprises a deletion, addition or substitution in the region between and including nucleotides 24 and 28 of SEQ ID NO:2.

8. The method of claim 1, wherein the *ica* regulatory nucleic acid comprises a five nucleotide non-wildtype substitution between and including nucleotides 24 and 28 of SEQ ID NO:2.

9. The method of claim 8, wherein the five nucleotide non-wildtype substitution has a sequence of ATAAA.

10. A method of making a polysaccharide over-producing bacterium comprising

introducing into a bacterium an *ica* nucleic acid operably linked to an *ica* regulatory nucleic acid, wherein the *ica* regulatory nucleic acid comprises a mutant *icaR* nucleic acid, and

measuring polysaccharide production from the bacterium, wherein a high level of polysaccharide production is indicative of a polysaccharide over-producing bacterium.

11. The method of claim 10, wherein the bacterium is a *Staphylococcus* bacterium.

12. The method of claim 11, wherein the *Staphylococcus* bacterium is selected from the group consisting of *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Staphylococcus capitis, Staphylococcus caprae, Staphylococcus hemolyticus, Staphylococcus auricularis, Staphylococcus intermedius, Staphylococcus lugdunensis, Staphylococcus pasteuri,* and *Staphylococcus piscifermentans.*

13. The method of claim 10, wherein the mutant *icaR* nucleic acid does not encode a wildtype IcaR protein.

14. The method of claim 10, wherein the mutant *icaR* nucleic acid comprises a frameshift mutation relative to a wildtype *icaR* nucleic acid.

15. The method of claim 10, wherein the mutant *icaR* nucleic acid encodes a truncated IcaR protein.

16. The method of claim 10, wherein the mutant *icaR* nucleic acid encodes a mutant IcaR protein that binds to a target less efficiently than wildtype IcaR protein.

17. The method of claim 10, wherein the polysaccharide is PNAG.

18. A method of making a polysaccharide over-producing bacterium comprising recombinantly down-regulating wildtype IcaR protein production, and selecting a polysaccharide over-producing bacterium.

19. The method of claim 18, wherein wildtype IcaR protein production is at a level lower than in a wildtype bacterium.

20. The method of claim 18, wherein wildtype IcaR protein production in the bacterium is zero.

21. The method of claim 18, wherein the bacterium produces a mutant IcaR protein.

22. The method of claim 21, wherein the mutant IcaR protein binds to a target less efficiently than wildtype IcaR protein.

23. The method of claim 21, wherein the mutant IcaR protein is a truncated IcaR protein.

24. The method of claim 18, wherein the bacterium is a *Staphylococcus* bacterium.

25. The method of claim 24, wherein the *Staphylococcus* bacterium is selected from the group consisting of *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Staphylococcus capitis*, *Staphylococcus caprae*, *Staphylococcus hemolyticus*, *Staphylococcus auricularis*, *Staphylococcus intermedius*, *Staphylococcus lugdunensis*, *Staphylococcus pasteuri*, and *Staphylococcus piscifermentans*.

26. The method of claim 18, further comprising measuring polysaccharide production from the bacterium, wherein a high level of polysaccharide production is indicative of a polysaccharide over-producing bacterium.

27. A method of making a polysaccharide over-producing bacterium comprising

recombinantly altering the TATTT nucleotide sequence in the *ica* promoter region.

28. The method of claim 27, wherein the bacterium is a *Staphylococcus* bacterium.

29. The method of claim 28, wherein the *Staphylococcus* bacterium is selected from the group consisting of *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Staphylococcus capitis, Staphylococcus caprae, Staphylococcus hemolyticus, Staphylococcus auricularis, Staphylococcus intermedius, Staphylococcus lugdunensis, Staphylococcus pasteuri,* and *Staphylococcus piscifermentans.*

30. The method of claim 27, further comprising measuring polysaccharide production from the bacterium, wherein a high level of polysaccharide production is indicative of a polysaccharide over-producing bacterium.

31. The method of claim 27, wherein the TATTT nucleotide sequence is deleted.

32. The method of claim 27, wherein the TATTT nucleotide sequence is substituted with a five nucleotide non-wildtype nucleotide sequence.

33. The method of claim 32, wherein the five nucleotide non-wildtype substitution has a sequence of ATAAA.

34. A recombinant polysaccharide over-producing bacterium comprising an *ica* nucleic acid operably linked to an *ica* regulatory nucleic acid,

wherein the *ica* regulatory nucleic acid comprises

(a) nucleic acid molecules which hybridize under stringent conditions to a nucleic acid molecule having a sequence of SEQ ID NO:2, have an addition, deletion or substitution in a region between and including nucleotides 9 and 43 of SEQ ID NO:2, and that enhance production of a polysaccharide from an *ica* locus, and

(b) complements thereof.

wherein the bacterium is not MN8m.

35. The bacterium of claim 34, wherein the *ica* regulatory nucleic acid comprises the nucleotide sequence of SEQ ID NO:1.

36. The bacterium of claim 34, wherein the *ica* regulatory nucleic acid comprises the nucleotide sequence between and including nucleotides 9 and 38 of SEQ ID NO:1.

37. The bacterium of claim 34, wherein the *ica* regulatory nucleic acid comprises a deletion, addition or substitution in the region between and including nucleotides 24 and 28 of SEQ ID NO:2.

38. The bacterium of claim 34, wherein the *ica* regulatory nucleic acid comprises a five nucleotide non-wildtype substitution between and including nucleotides 24 and 28 of SEQ ID NO:2.

39. The bacterium of claim 38, wherein the five nucleotide non-wildtype substitution has a sequence of ATAAA.

40. The bacterium of claim 34, wherein the bacterium is a *Staphylococcus* bacterium.

41. The bacterium of claim 40, wherein the *Staphylococcus* bacterium is selected from the group consisting of *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Staphylococcus capitis*, *Staphylococcus caprae*, *Staphylococcus hemolyticus*, *Staphylococcus auricularis*, *Staphylococcus intermedius*, *Staphylococcus lugdunensis*, *Staphylococcus pasteuri*, and *Staphylococcus piscifermentans*.

42. A recombinant polysaccharide over-producing bacterium comprising a mutant *icaR* nucleic acid.

43. The bacterium of claim 42, wherein the mutant *icaR* nucleic acid is a deletion of wildtype *icaR* nucleic acid.

44. The bacterium of claim 42, wherein the mutant *icaR* nucleic acid encodes a mutant IcaR protein.

45. The bacterium of claim 42, wherein the mutant IcaR protein binds to a target less efficiently than wildtype IcaR protein.

46. The bacterium of claim 42, wherein the mutant IcaR protein is a truncated IcaR protein.

47. The bacterium of claim 42, wherein the bacterium is a *Staphylococcus* bacterium.

48. The bacterium of claim 47, wherein the *Staphylococcus* bacterium is selected from the group consisting of *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Staphylococcus capitis, Staphylococcus caprae, Staphylococcus hemolyticus, Staphylococcus auricularis, Staphylococcus intermedius, Staphylococcus lugdunensis, Staphylococcus pasteuri,* and *Staphylococcus piscifermentans.*

49. A method of producing a bacterial polysaccharide comprising

culturing the polysaccharide over-producing bacterium of claim 34-47 or 48 in a growth medium, and

harvesting the bacterial polysaccharide from the culture.

50. The method of claim 49, wherein the bacterial polysaccharide is composed of β 1-6 linked glucosamine units, and wherein 0-100% of the units are acetate substituted.

51. The method of claim 50, wherein less than 50% of the units are acetate substituted.

52. The method of claim 49, further comprising formulating the bacterial polysaccharide as a vaccine.

53. A method of producing an antibody to a bacterial polysaccharide comprising

isolating a bacterial polysaccharide from the polysaccharide over-producing bacterium of claim 34-47 or 48,

administering to a subject the isolated bacterial polysaccharide in an amount effective to produce an antibody, and

harvesting antibody from the subject.

54. The method of claim 53, further comprising isolating the antibody.

55. The method of claim 53, wherein the subject is a non-human subject.

56. The method of claim 53, wherein the antibody is a polyclonal antibody.

57. The method of claim 55, wherein the non-human subject is a rabbit or a mouse.

58. The method of claim 53, further comprising administering an adjuvant to the subject.

59. The method of claim 53, further comprising harvesting an antibody-producing cell from the subject and harvesting the antibody from the antibody-producing cell.

60. The method of claim 59, wherein the antibody-producing cell is manipulated prior to antibody harvest.

61. The method of claim 53, wherein the isolated bacterial polysaccharide is composed of β 1-6 linked glucosamine units, and wherein 0-100% of the units are acetate substituted.

62. The method of claim 61, wherein less than 50% of the units are acetate substituted.

63. An isolated nucleic acid molecule, comprising

(a) nucleic acid molecules which hybridize under stringent conditions to a nucleic acid molecule having a sequence of SEQ ID NO:2, have an addition, deletion or substitution in a region between and including nucleotides 9 and 43 of SEQ ID NO:2, and that enhance production of a polysaccharide from an *ica* locus, and

(b) complements thereof.

64. The isolated nucleic acid molecule of claim 63, wherein the isolated nucleic acid molecule comprises a sequence of SEQ ID NO:1.

65. The isolated nucleic acid molecule of claim 63, wherein the isolated nucleic acid molecule comprises a nucleotide sequence between and including nucleotides 9 and 38 of SEQ ID NO:1.

66. The isolated nucleic acid molecule of claim 63, wherein the isolated nucleic acid molecule comprises a deletion, addition or substitution in the region between and including nucleotides 24 and 28 of SEQ ID NO:2.

67. The isolated nucleic acid molecule of claim 63, wherein the isolated nucleic acid molecule comprises a five nucleotide non-wildtype substitution between and including nucleotides 24 and 28 of SEQ ID NO:2.

68. The isolated nucleic acid molecule of claim 67, wherein the five nucleotide non-wildtype substitution has a sequence of ATAAA.

69. An expression vector comprising the isolated nucleic acid molecule of claim 63-67 or 68, operably linked to an *ica* nucleic acid.

70. A host cell transformed or transfected with the expression vector of claim 69.

71. An isolated nucleic acid molecule selected from the group consisting of
(a) a fragment of a nucleic acid molecule having a sequence of SEQ ID NO:1, and
(b) complements of (a),
wherein the fragment spans a MN8m mutation and enhances production of a polysaccharide from an *ica* locus when operably linked to an *ica* nucleic acid.

72. The isolated nucleic acid molecule of claim 71, wherein the fragment has a nucleotide sequence between and including nucleotides 9 and 38 of SEQ ID NO:1.

73. A method for identifying an isolated binding agent, comprising
contacting a first nucleic acid molecule having the sequence of SEQ ID NO:2 or a functionally equivalent fragment thereof with a candidate molecule and determining whether the candidate molecule binds to the first nucleic acid molecule, and

contacting a second nucleic acid molecule having the sequence of SEQ ID NO:1 or a functionally equivalent fragment thereof with the candidate molecule and determining whether the candidate molecule binds to the second nucleic acid molecule,

wherein a candidate molecule that binds to either the first or the second nucleic acid molecule but not both is indicative of an isolated binding agent.

74. The method of claim 73, further comprising contacting a reporter construct containing the first nucleic acid molecule with the candidate molecule to determine if the candidate molecule is a polysaccharide synthesis modulator, wherein the candidate molecule is a polysaccharide synthesis modulator if expression from the reporter construct is altered.

75. The method of claim 73, wherein the candidate molecule is a nucleic acid molecule.

76. The method of claim 73, wherein the candidate molecule is a peptide.

77. The method of claim 73, wherein the candidate molecule is a small molecule.

78. The method of claim 77, wherein the small molecule is a library member.

79. The method of claim 73, wherein the candidate molecule is conjugated to a detectable label.

80. The method of claim 79, wherein the detectable label is selected from the group consisting of a radioactive label, an enzyme, a biotin molecule, an avidin molecule or a fluorochrome.

81. The method of claim 73, wherein the candidate molecule is conjugated to a cytotoxic agent.

82. The method of claim 73, wherein the candidate molecule inhibits transcription of an *ica* nucleic acid molecule upon binding selectively to the nucleic acid molecule having a sequence of SEQ ID NO:2.

83. The method of claim 73, wherein the candidate molecule inhibits transcription of an *ica* nucleic acid molecule upon binding selectively to the nucleic acid molecule having a nucleotide sequence of SEQ ID NO:1.

84. A method of identifying an *ica* promoter sequence associated with polysaccharide overproduction comprising

detecting a nucleic acid molecule having a sequence alteration from wildtype in a region between and including nucleotides 9 and 43 of SEQ ID NO:2.

85. The method of claim 84, wherein the nucleic acid molecule is detected by contacting a candidate nucleic acid with a first and a second nucleic acid amplification primer, wherein at least one of the first or second nucleic acid amplification primers is capable of hybridizing to a sequence of SEQ ID NO:1 and not capable of hybridizing to a sequence of SEQ ID NO:2,

amplifying a primed nucleic acid molecule which hybridizes to the first and the second nucleic acid amplification primers, and

detecting the presence of an amplified nucleic acid molecule.

86. The method of claim 84, wherein the nucleic acid molecule is detected by contacting a candidate nucleic acid with a first and a second nucleic acid amplification primer, wherein the first and second nucleic acid amplification primers respectively hybridize to nucleotide sequences 5' and 3' to nucleotides 24 and 28 of SEQ ID NO:2,

amplifying a primed nucleic acid molecule which hybridizes to the first and the second nucleic acid amplification primers, and

measuring and comparing the length of the amplified nucleic acid molecule to a first control amplified nucleic acid molecule comprising SEQ ID NO:1 or a second control amplified nucleic acid molecule comprising SEQ ID NO:2,

wherein an amplified nucleic acid molecule that is

(a) identical in length to a first control amplified nucleic acid molecule or

(b) shorter than the second control amplified nucleic acid molecule

is indicative of the presence of an *ica* promoter sequence associated with polysaccharide over-production.

87. The method of claim 84, wherein the nucleic acid molecule is detected by

contacting a candidate nucleic acid with a nucleic acid probe that selectively binds to SEQ ID NO:1 and does not bind to SEQ ID NO:2, and

detecting the presence of the bound probe.

88. The method of claim 85, 86 or 87, wherein the candidate nucleic acid is present in a bacterial isolate from a subject.

89. The method of claim 85, 86 or 87, wherein the candidate nucleic acid is present in a bacterial culture.

90. A method for identifying an *ica* regulatory nucleic acid molecule that enhances polysaccharide production comprising

altering a nucleic acid molecule having a sequence of SEQ ID NO:2, and

determining a level of reporter production by a bacterium that comprises the altered nucleic acid molecule operably linked to reporter nucleic acid,

wherein a higher than wildtype level of reporter protein production is indicative of an *ica* regulatory nucleic acid molecule that enhances polysaccharide production.

91. The method of claim 90, wherein the reporter nucleic acid is an *ica* nucleic acid and reporter production is polysaccharide production.

92. The method of claim 90, wherein the nucleic acid molecule is altered recombinantly.

93. The method of claim 90, wherein the nucleic acid molecule is altered naturally during bacterial culture.

94. The method of claim 90, wherein the bacterium is a *Staphylococcus* bacterium.

95. The method of claim 90, wherein the *Staphylococcus* bacterium is selected from the group consisting of *Staphylococcus epidermidis, Staphylococcus aureus, Staphylococcus capitis, Staphylococcus caprae, Staphylococcus hemolyticus, Staphylococcus auricularis, Staphylococcus intermedius, Staphylococcus lugdunensis, Staphylococcus pasteuri,* and *Staphylococcus piscifermentans.*

96. A composition comprising

an isolated binding agent that binds to a nucleic acid having a sequence of SEQ ID NO:1 with greater affinity than to SEQ ID NO:2.

97. A composition comprising

an isolated binding agent that binds to a nucleic acid having a sequence of SEQ ID NO:2 with greater affinity than to SEQ ID NO:1.

98. The composition of claim 96 or 97, wherein the isolated binding agent is a nucleic acid molecule.

99. The composition of claim 96 or 97, wherein the isolated binding agent is a peptide.

100. The composition of claim 96 or 97, wherein the isolated binding agent is a small molecule.

101. The composition of claim 96 or 97, wherein the isolated binding agent is a library member.

102. The composition of claim 96 or 97, wherein the isolated binding agent is conjugated to a detectable label.

103. The composition of claim 102, wherein the detectable label is selected from the group consisting of a radioactive label, an enzyme, a biotin molecule, an avidin molecule or a fluorochrome.

104. The composition of claim 96 or 97, wherein the isolated binding agent is conjugated to a cytotoxic agent.

105. The composition of claim 96, wherein the isolated binding agent inhibits transcription of an *ica* nucleic acid molecule upon binding selectively to the nucleic acid molecule having a nucleotide sequence of SEQ ID NO:1.

106. The composition of claim 97, wherein the isolated binding agent inhibits transcription of an *ica* nucleic acid molecule upon binding selectively to the nucleic acid molecule having a sequence of SEQ ID NO:2.

107. A method of over-producing a protein in a bacterium comprising

introducing into a bacterium a nucleic acid operably linked to an *ica* regulatory nucleic acid,

wherein the *ica* regulatory nucleic acid comprises

(a) nucleic acid molecules which hybridize under stringent conditions to a nucleic acid molecule having a sequence of SEQ ID NO:2, have an addition, deletion or substitution in a region between and including nucleotides 9 and 43 of SEQ ID NO:2, and that enhance production of a polysaccharide from an *ica* locus, and

(b) complements thereof, and

wherein the nucleic acid encodes a protein to be over-produced.

108. The method of claim 107, wherein the bacterium is a *Staphylococcus* bacterium.

109. The method of claim 108, wherein the *Staphylococcus* bacterium is selected from the group consisting of *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Staphylococcus capitis*, *Staphylococcus caprae*, *Staphylococcus hemolyticus*, *Staphylococcus auricularis*, *Staphylococcus intermedius*, *Staphylococcus lugdunensis*, *Staphylococcus pasteuri*, and *Staphylococcus piscifermentans*.

110. The method of claim 107, further comprising measuring protein production from the bacterium, wherein a high level of protein production is indicative of an over-producing bacterium.

111. The method of claim 107, wherein the *ica* regulatory nucleic acid comprises the nucleotide sequence of SEQ ID NO:1.

112. The method of claim 107, wherein the *ica* regulatory nucleic acid comprises the nucleotide sequence between and including nucleotides 9 and 38 of SEQ ID NO:1.

113. The method of claim 107, wherein the *ica* regulatory nucleic acid comprises a deletion, addition or substitution in the region between and including nucleotides 24 and 28 of SEQ ID NO:2.

114. The method of claim 107, wherein the *ica* regulatory nucleic acid comprises a five nucleotide non-wildtype substitution between and including nucleotides 24 and 28 of SEQ ID NO:2.

115. The method of claim 114, wherein the five nucleotide non-wildtype substitution has a sequence of ATAAA.

116. A method of over-producing a protein in a bacterium comprising

introducing into a bacterium a nucleic acid operably linked to an *ica* regulatory nucleic acid, wherein the *ica* regulatory nucleic acid comprises a mutant *icaR* nucleic acid,

wherein the nucleic acid encodes a protein to be over-produced.

117. The method of claim 116, wherein the bacterium is a *Staphylococcus* bacterium.

118. The method of claim 117, wherein the *Staphylococcus* bacterium is selected from the group consisting of *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Staphylococcus capitis, Staphylococcus caprae, Staphylococcus hemolyticus, Staphylococcus auricularis, Staphylococcus intermedius, Staphylococcus lugdunensis, Staphylococcus pasteuri,* and *Staphylococcus piscifermentans.*

119. The method of claim 116, wherein the mutant *icaR* nucleic acid does not encode a wildtype IcaR protein.

120. The method of claim 116, wherein the mutant *icaR* nucleic acid comprises a frameshift mutation relative to a wildtype *icaR* nucleic acid.

121. The method of claim 116, wherein the mutant *icaR* nucleic acid encodes a truncated IcaR protein.

122. The method of claim 116, wherein the mutant *icaR* nucleic acid encodes a mutant IcaR protein that binds to a target less efficiently than wildtype IcaR protein.

123. The method of claim 10 or 116, wherein the *ica* regulatory nucleic acid further comprises an *ica* promoter.

124. The method of claim 107 or 116, wherein the protein is a therapeutic protein.

125. The method of claim 107 or 116, wherein the protein is a fluorescent protein.

126. The method of claim 125, wherein the fluorescent protein is selected from the group consisting of green fluorescent protein, yellow fluorescent protein and cyan fluorescent protein.

127. The method of claim 107 or 116, wherein the protein is a bacterial protein.

128. The method of claim 127, wherein the bacterial protein is Staphylococcal clumping factor A, Staphylococcal clumping factor B, Staphylococcal protein A, or Streptococcal protein G.

129. The method of claim 107 or 116, wherein the protein is a mammalian protein.

130. The method of claim 107 or 116, wherein the protein is a viral protein, a fungal protein or a parasite protein.

131. The method of claim 107 or 116, wherein the protein is fibrinogen-binding protein.

132. The method of claim 107 or 116, wherein the protein is a vaccine carrier protein.

133. The method of claim 107 or 116, wherein the protein is IcaA, IcaD, IcaB or IcaC.